Sister chromatid separation: **Falling apart at the seams**

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Cohesion between sister chromatids must be dissolved at the time of chromosome segregation. Recent studies reveal that the principles of cohesion dissolution in mitosis and meiosis are the same, but that there are important differences that stem from the distinct natures of these two processes.

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One of the key processes in cell division is the precise segregation of chromosomes. In mitosis, cohesion between the two sister chromatids allows the formation of bipolar spindle attachments (Figure 1), ultimately segregating the sister chromatids to the two daughter cells. In meiosis, cells must keep track of homologous chromosomes, which separate at the first meiotic division in what is known as reductional segregation, and of sister chromatids, which separate only at the second meiotic division in what is known as equational segregation (Figure 1). In the complete absence of cohesion, chromosomes segregate at random, often with dire consequences to the cell. However, for the chromosomes to physically separate, cohesion must be dissolved. Clearly the timing of cohesion dissolution is critical, and in meiosis at least some cohesion must remain after the first meiotic division to ensure proper sister chromatid segregation at the second meiotic division. Recent studies have revealed that, while the dissolution of cohesion occurs by a similar mechanism in meiosis and mitosis, the process in meiosis has an added twist.

Cohesion: variations on a theme

Cohesion between sister chromatids is mediated by a protein complex named cohesin that contains at least four conserved subunits (reviewed in [1,2]). In the budding yeast *Saccharomyces cerevisiae*, these subunits are Smc1, Smc3, Scc3 and Scc1/Mcd1. In the fission yeast *Schizosaccharomyces pombe*, the fruitfly *Drosophila* and the frog *Xenopus*, the Scc1/Mcd1 homologue is called Rad21. Vertebrates have two distinct cohesin complexes, which both contain Smc1, Smc3 and Scc1/Mcd1, but that differ in their Scc3 orthologue, which is either SA1 or SA2 [3,4]. Whether these two complexes serve different functions is not known. An additional protein, Pds5 (known as Spo76 in *Sordaria* and BimD in *Aspergillus nidulans*), has recently been suggested to also be a structural component of the cohesion apparatus in fungi and animal cells (reviewed in [1,2]).

Where do cohesins bind? Immunolocalization in animal and yeast cells revealed that cohesins bind at the centromeric region and at multiple sites along chromosome arms. The fine mapping of cohesin-binding sites in yeast showed that cohesins bind chromosome arms at approximately 10 kilobase intervals, at sites that tend to be AT-rich (reviewed in [1]). The cohesin-binding sites in the vicinity of the centromere are somewhat different, requiring centromeric DNA elements that also function in the establishment of the kinetochore [1]. These centromeric elements are not present at the cohesin-binding sites on the chromosome arms, suggesting that arm cohesion and centromeric cohesion may be different. This indeed turns out to be the case (see below).

The dissolution of cohesion: when breakup is a good thing

At the time of chromosome segregation, cohesion between sister chromatids must be dissolved. This is true not only in mitosis and the second meiotic division (meiosis II), but also in the first meiotic division (meiosis I). The requirement for complete cohesion dissolution in mitosis and meiosis II is obvious: any residual cohesion between the two sisters would counteract the bipolar pulling forces of the spindle (Figure 1). But why is it necessary to dissolve cohesion in meiosis I, when the two sister chromatids segregate to the same pole? The answer lies in the mechanism that directs pairing of homologous chromosomes, an essential step for the subsequent segregation of the homologues.

During meiosis I, homologous chromosomes recombine and form a crossover, or chiasma (Figure 1b, reviewed in [5]). It is believed that cohesion distal to the chiasma is necessary to prevent the chiasma from sliding off the ends of the chromosomes, resulting in the loss of homologue pairing. At anaphase of meiosis I, sister chromatid arms must separate, or the two homologues will stay attached (Figure 1b). One might expect that, if sister chromatid cohesion completely dissolves in meiosis I, the sister chromatids would not be able to segregate equationally at meiosis II. Thus, some form of cohesion between the sister chromatids must be maintained until metaphase of meiosis II.

In mitosis, coincident with sister chromatid separation, at least two of the cohesin subunits, Scc3 and Scc1/Mcd1, dissociate from chromosomes. This dissociation depends on the activity of a conserved enzyme, referred to as ‘separin’, originally named Esp1 in *S. cerevisiae* and Cut1 in *S. pombe* (Figure 1, [1,2]). The dissociated Scc1/Mcd1 is cleaved at two sites in a separin-dependent manner [6]. Mutating the two cleavage sites of Scc1/Mcd1 resulted in

a non-cleavable protein that failed to dissociate from chromatin and inhibited sister chromatid separation *in vivo* [6]. Sequence comparisons revealed that separins have a domain resembling the active site of a subset of cysteine proteases [7]. Based on sequence alignment, the amino acids in the putative separin active site were identified; inactivation of these residues abolished the ability of purified separin to cleave Scc1/Mcd1 *in vitro* [7].

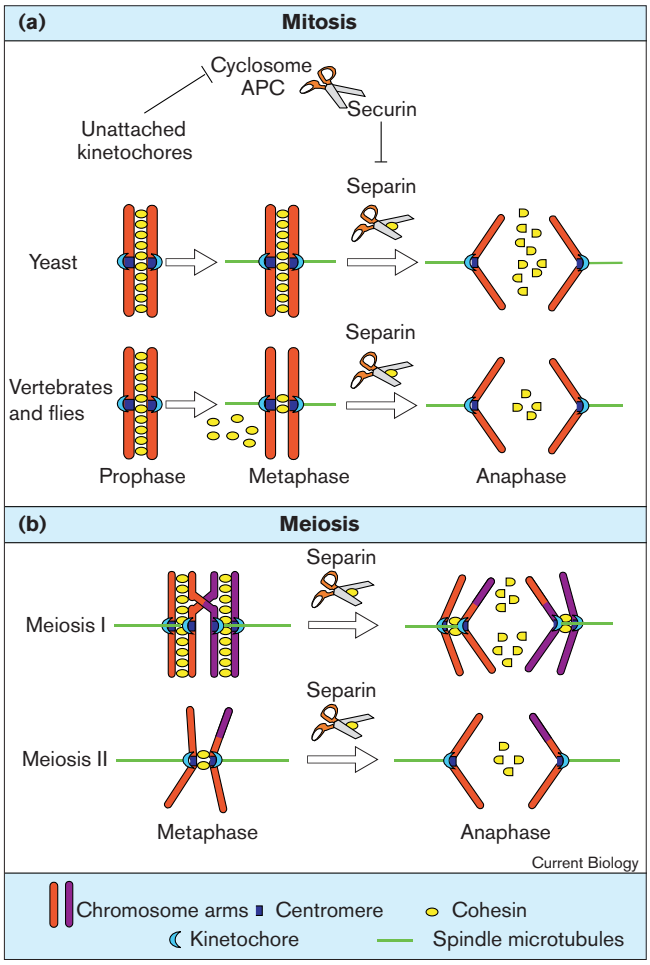
Taken together, these results strongly suggest that, in mitosis, sister chromatid separation is mediated by the proteolytic cleavage of Scc1/Mcd1 by separin. Whether separins have additional substrates is currently not known. By using a recombinant Scc1/Mcd1 containing a cleavage site for the tobacco etch virus (TEV) protease, Uhlmann *et al.* [7] were able to show that TEV-induced Scc1/Mcd1 cleavage is sufficient to trigger sister chromatid separation, even in the absence of separin function.

Unlike in yeast, where cohesins dissociate from chromosomes at the onset of anaphase, in vertebrates and flies most of the cohesin complex dissociates from chromosomes in prophase, and only a small amount of cohesin can be detected at centromeric regions [8,9]. While it cannot be ruled out that undetectable cohesin complexes are still present along chromosome arms, arm separation can be clearly observed when cells are arrested in prometaphase under conditions which should inhibit separin activity (reviewed in [10]). Still, the presence of separins in animal cells and the retention of centromeric cohesion suggested that a separin-dependent mechanism for cohesion dissolution does exist.

In animal cells, cohesion dissolution may thus occur in two steps: a separin-independent step in prophase for dissolution of arm cohesion, and a separin-dependent step in anaphase for dissolution of centromeric cohesion. The nature of the molecular difference between arm and centromeric cohesion, or the feature that protects centromeric cohesion when arm cohesion is dissolved, is currently not known. In flies, the MEI-S332 protein localizes to centromeric regions in both mitosis [11] and meiosis (see below), and in its absence sister chromatids separate prematurely, suggesting that MEI-S332 may be involved in maintaining centromeric cohesion. The difference in cohesin dissociation between yeast and animal cells may have to do with the much higher degree of condensation that chromosomes in animal cells undergo, which may be incompatible with a high density of cohesins.

In meiosis of both budding and fission yeast, the role of Scc1/Mcd1 and Rad21, respectively, is taken over by the meiosis-specific protein Rec8 [12,13]. Rec8 dissociates from chromosome arms in meiosis I, thereby allowing homologue separation, but it remains at the centromeres until anaphase of meiosis II, ensuring proper sister chromatid segregation at that stage [12,13]. As with its mitotic counterpart, Rec8 is also cleaved by separin [14]. Mutating the Rec8 cleavage sites was found to abolish the separin-dependent cleavage, and to prevent both homologue and sister chromatid separation [14]. While Rec8 can substitute

Figure 1



Chromosome segregation in meiosis and mitosis. **(a)** Yeast (top) retain their arm cohesion until metaphase, whereas vertebrates and flies (bottom) only maintain centromeric cohesion. In metaphase, the chromosomes are attached to spindle microtubules in a bipolar fashion, via kinetochores which assemble at the chromosome's centromere. At the metaphase-to-anaphase transition, cohesins are cleaved by separin and leave the DNA, thereby allowing the chromatids to segregate to opposite poles. The inhibitor of separin, securin, is degraded only when all kinetochores have formed stable bipolar spindle attachment (see text for details). **(b)** In meiosis I, the chiasmata physically link homologous chromosomes. Note that, as long as there is cohesion between the sister chromatids, the homologues cannot separate. The two sister kinetochores act in concert and bind microtubules in a monopolar fashion. However, kinetochores of the two homologues are bound to opposite poles. At anaphase I, only arm cohesion is lost and the two homologues segregate to opposite poles (reductional segregation). In meiosis II, the sister kinetochores bind spindle microtubules in a bipolar fashion. The remaining cohesion is lost and the sister chromatids separate equationally. Both meiosis I and meiosis II are regulated by securin (not shown).

for Scc1/Mcd1 or Rad21 in mitosis [13,14], Rad21 in fission yeast, at least, cannot substitute for Rec8 in meiosis [13], suggesting that Rec8 has meiosis-specific functions that Rad21 lacks.

Cells undergoing meiosis I in the absence of Rec8 exhibit equational, rather than reductional chromosome segregation [12,13], implying that, in *rec8* mutant cells, sister chromatids separate completely in meiosis I, and that their kinetochores bind spindle microtubules in a bipolar fashion, as in mitosis. Premature sister chromatid separation in the first meiotic division was also observed in the budding yeast *slk19* mutant and in the *Drosophila mei-S332* mutant [15,16]. In the *slk19* mutant, chromosomes segregate equationally at the first meiotic division and show a significant reduction in centromeric Rec8 in anaphase of meiosis I [15,17]. It is therefore possible that Slk19 and MEI-S332, both of which localize to centromeric regions [15–17], act as 'guardians' of centromeric cohesion, preventing the premature dissociation of Rec8.

These results further suggest that Rec8, and perhaps centromeric cohesion *per se*, is essential not only for maintaining sister chromatid association, but also for monopolar spindle binding in meiosis I. What causes kinetochores to change their behavior from monopolar in meiosis I to bipolar in meiosis II is not clear. Recent studies using grasshopper spermatocytes suggest that meiotic kinetochores are technically capable of bipolar spindle attachments as early as anaphase of meiosis I, when centromeric cohesion is still intact [18]. Thus, the exact relationship between kinetochore polarity and centromeric cohesion remains to be determined.

Regulation of cohesion dissolution: timing is everything

To ensure proper chromosome segregation in mitosis, sister chromatids must separate only after the establishment of bipolar spindle attachments. The timing at which cohesion is dissolved is regulated, at least in part, by the presence of a separin inhibitor named securin (Pds1 in *S. cerevisiae*, Cut2 in *S. pombe* and PTTG, for pituitary tumor transforming gene, in humans, Figure 1a). Securins physically associate with separins [19,20], and separin activation occurs only after securin is degraded. The degradation of securin occurs at the metaphase-to-anaphase transition, in a process that involves the cyclosome/anaphase-promoting complex (APC, [21,22]). The cyclosome/APC is activated only after all chromosomes have established stable spindle attachments (Figure 1a). Thus, separin becomes active only when all chromosomes are ready for segregation.

Securins also appear to have a positive role in separin activation, in that they are required for proper nuclear localization of separins [23]. This safeguard mechanism ensures that separins will not wander by themselves into the nucleus and activate sister chromatid separation prematurely. Securins are also present in both meiotic divisions [24], presumably acting then in the same way as they do in mitosis. In budding yeast, and perhaps in other organisms, additional regulatory mechanisms must exist, as even in the absence of securins the dissolution of cohesion does not happen prematurely [14,20]. Scc1/Mcd1 was found to be phosphorylated shortly before its cleavage, and separin is more efficient in cleaving the phosphorylated form of Scc1/Mcd1 [7]. The *Xenopus* SA1 subunit was also found to be phosphorylated [4]. It is therefore possible that both securin degradation and the phosphorylation of cohesin together control the timing of cohesion dissolution.

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